

This listing of claims will replace all prior versions of claims in the application.

Claim 1. (original) A method for identifying the presence of a BBB-specific protein or fragment thereof in endothelial cells of brain capillaries, ~~comprising~~ characterized in that

- a) endothelial of brain capillaries freshly isolated from brain are conventionally pre-purified by enzymatic digestion,
- b) the digest obtained in step a) is treated with a lysis buffer that essentially destroys erythrocytes and apoptotic cells present and maintains at least 70% of the endothelial cells of brain capillaries in vital form,
- c) the product obtained in step b) is optionally purified further,
- d) a subtractive cDNA library is prepared from the endothelial cells of brain capillaries and a subtractive tissue,
- e) a cDNA subtraction is performed using one or more differential hybridization(s),
- f) clones from the subtractive cDNA library are verified by differential hybridization with respect to their respective expression,
- g) the cDNA sequence is completed for the BBB-specific clones from the subtractive cDNA library and
- h) the expression pattern of the investigated clones is compared between fresh and cultured endothelial cells of brain capillaries and, that way, the presence of BBB-specific proteins or fragments thereof is identified.

Claim 2. (currently amended) The method according to claim 1, ~~wherein characterized in that~~ the lysis buffer in step b) has the following composition:

Na^+	30.0 mM to	60.0 mM
K^+	5.0 mM to	7.5 mM
NH_4^+	80.0 mM to	100.0 mM
Ca^{2+}	1.0 mM to	2.0 mM
Mg^{2+}	6.0 mM to	9.0 mM
Cl^-	125.0 mM to	175.0 mM
HCO_3^-	4.5 mM to	6.5 mM
H_2PO_4^-	0.5 mM to	2.5 mM
SO_4^{2-}	0.3 mM to	0.6 mM
HPO_4^{2-}	0.4 mM to	0.7 mM
Glucose	1.5 mM to	3.0 mM

Claim 3. (currently amended) The method according to claim 2, ~~wherein characterized in that~~ the lysis buffer has the following composition:

NaCl	30 mM	to	50 mM
KCl	4.5 mM	to	5.5 mM

NH ₄ Cl	80 mM	to	100 mM
CaCl ₂	1.0 mM	to	2.0 mM
MgCl ₂	0.6 mM	to	0.8 mM
MgSO ₄	0.3 mM	to	0.6 mM
NaHCO ₃	4.5 mM	to	6.5 mM
NaH ₂ PO ₄	0.2 mM	to	0.45 mM
Na ₂ HPO ₄	0.4 mM	to	0.65 mM
KH ₂ PO ₄	0.1 mM	to	0.15 mM
Glucose	1.5 mM	to	3.0 mM

Claim 4. (currently amended) The method ~~according to claim 1 wherein according to one of claims 1 to 3,~~ characterized in that the subtractive tissue in step f) are aortic endothelial cells.

Claim 5. (currently amended) The method according to ~~claim 1 wherein one of claims 1 to 4,~~ characterized in that the complete cDNA sequence in step i) is prepared by screening cDNA libraries and RACE-PCR.

Claim 6. (currently amended) The method according to ~~claim 1 one of claims 1 to 5,~~ characterized in that the endothelial cells of brain capillaries are derived from man or pig.

Claim 7. (currently amended) A protein with BBB-specificity or a fragment thereof, obtainable according to a method according to ~~claim 1 one of the claims 1 to 6.~~

Claim 8. (currently amended) The protein according to claim 7, wherein the

protein comprises a ~~characterized in that it has a~~ sequence selected from SEQ ID NO: 5, SEQ ID NO: 14, SEQ ID NO: 19, or SEQ ID NO: 53.

Claim 9. (currently amended) A method for the identification of a presence of a BBB-specific protein or fragment thereof in endothelial cells of brain capillaries, comprising ~~characterized in that~~

- a) endothelial cells of brain capillaries freshly isolated from brain are conventionally pre-purified by enzymatic digestion,
- b) the digest obtained in step a) is treated with a lysis buffer that essentially destroys erythrocytes and apoptotic cells present and maintains at least 70% of the endothelial cells of brain capillaries in vital form,
- c) the product obtained in step b) is optionally purified further,
- d) the product obtained in step c) is solubilized in a suitable buffer,
- e) an isoelectric focusing is performed,
- f) the samples from the isoelectric focusing are separated in the second dimension according to the molecular weight,
- g) differential spots are identified and isolated,
- h) mass spectrometric analysis is performed with the isolate of g), and
- i) an evaluation thereof is constructed via specific database analysis.

Claim 10. (currently amended) The method according to claim 9, wherein
~~characterized in that~~ a lysis buffer in step b) has the following composition:

Na^+	30.0 mM to	60.0 mM
K^+	5.0 mM to	7.5 mM
NH_4^+	80.0 mM to	100.0 mM
Ca^{2+}	1.0 mM to	2.0 mM
Mg^{2+}	6.0 mM to	9.0 mM
Cl^-	125.0 mM to	175.0 mM
HCO_3^-	4.5 mM to	6.5 mM
H_2PO_4^-	0.5 mM to	2.5 mM
SO_4^{2-}	0.3 mM to	0.6 mM
HPO_4^{2-}	0.4 mM to	0.7 mM
Glucose	1.5 mM to	3.0 mM

Claim 11. (currently amended) The method according to claim 10, wherein
~~characterized in that~~ the lysis buffer has the following composition:

NaCl	30 mM	to	50 mM
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KCl	4.5 mM	to	5.5 mM
NH ₄ Cl	80 mM	to	100 mM
CaCl ₂	1.0 mM	to	2.0 mM
MgCl ₂	0.6 mM	to	0.8 mM
MgSO ₄	0.3 mM	to	0.6 mM
NaHCO ₃	4.5 mM	to	6.5 mM
NaH ₂ PO ₄	0.2 mM	to	0.45 mM
Na ₂ HPO ₄	0.4 mM	to	0.65 mM
KH ₂ PO ₄	0.1 mM	to	0.15 mM
Glucose	1.5 mM	to	3.0 mM

Claim 12. (currently amended) A protein with BBB-specificity or a fragment thereof, obtainable according to a method according to ~~claim 9 one of claims 9 to 11.~~

Claim 13. (currently amended) The protein according to claim 12, wherein the protein comprises a ~~characterized in that it has a sequence selected from SEQ ID NO: 23, SEQ ID NO: 27, SEQ ID NO: 33.~~

Claims 14-15. (cancelled)

Claim 16. (currently amended) An agent for the diagnosis of diseases that are based on the dysfunction of the blood-brain barrier, characterized in that it comprises a protein according to ~~claim 7 one of the claims 7 to 8 or 12 to 13.~~

Claim 17. (currently amended) The agent for the therapy of diseases which are based on a dysfunction of the blood-brain barrier, characterized in that it comprises a protein according ~~claim 7~~ ~~one of claims 7 to 8 or 12 to 13~~.

Claims 18-20. (cancelled)

Claim 21. (new) A method for identifying the presence of a BBB-specific protein or fragment thereof in endothelial cells of brain capillaries, comprising:

- a) purifying endothelial of brain capillaries,
- b) treating the digest obtained in step a) with a buffer that can essentially destroy erythrocytes and apoptotic cells present and maintain at least about 70% of the endothelial cells of brain capillaries in vital form,
- c) optionally purifying further the product obtained in step b) is optionally purified further,
- d) preparing a subtractive cDNA library from the endothelial cells of brain capillaries and a subtractive tissue,
- e) performing a cDNA subtraction,
- f) verifying clones from the subtractive cDNA library,
- g) completing the cDNA sequence for the BBB-specific clones from the subtractive cDNA library and

- h) comparing the expression pattern of the investigated clones between fresh and cultured endothelial cells of brain capillaries and thereby identifying the presence of BBB-specific proteins or fragments thereof.

Claim 22. (new) A method for diagnosis of a disease or condition associated with an ischemic condition, comprising use of one or more sequences that comprise SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 32, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, for the preparation of an agent for the diagnosis of diseases which are connected with ischemic conditions.

Claim 23. (new) The method according to claim 22 wherein the one or more sequences are used to diagnosis of stroke, myocardial infarction or tumor-associated conditions.

Claim 24. (new) The method of claim 22 wherein the diagnosis is carried out via the control of the expression of one or more polypeptides encoded by the one or more sequences.

Claim 25. (new) A method for transporting a substance through the blood-brain barrier comprising using a polypeptide of claim 7.

Claim 26. (new) A method for diagnosis or therapy associated with blood-brain barrier dysfunction comprising use of a protein of claim 7.